

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A23G 9/00, 9/02, A23L 3/37	A1	(11) International Publication Number: WO 98/41107 (43) International Publication Date: 24 September 1998 (24.09.98)
(21) International Application Number: PCT/EP98/01576 (22) International Filing Date: 12 March 1998 (12.03.98) (30) Priority Data: 97301719.7 14 March 1997 (14.03.97) EP <i>(34) Countries for which the regional or international application was filed:</i> GB et al. (71) Applicant (for all designated States except AU BB CA GB IE KE LK LS MN MW NZ SD SG SZ TT UG): UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL). (71) Applicant (for AU BB CA GB IE KE LK LS MN MW NZ SD SG SZ TT UG only): UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB). (72) Inventor: SMALLWOOD, Keith; Unilever Research Colworth, Colworth House, Sharnbrook MK44 1LQ (GB). (74) Agent: UNILEVER N.V.; Patent Division, P.O. Box 137, NL-3130 AC Vlaardingen (NL).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: FROZEN FOOD PRODUCT (57) Abstract Use of storage temperatures of from -2 °C to -12 °C for frozen food products containing anti-freeze proteins.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

Frozen Food productTechnical Field of the Invention

5 The invention relates to a method of storing food product containing AFPs and to food products containing AFPs.

Background to the Invention

10 Anti-freeze peptides (AFP) have been suggested for improving the freezing tolerance of foodstuffs.

For the purpose of this invention the term AFP has the meaning such as well-known in the art, see for example
15 "Antifreeze proteins and their potential use in frozen food products", Marilyn Griffiths et al. Biotechnology Advances, Vol.13, pp.375-402, 1995.

WO 90/13571 discloses antifreeze peptides produced
20 chemically or by recombinant DNA techniques from plants. The AFPs can suitably be used in food-products such as ice-cream.

WO 92/22581 discloses AFPs from plants which can be used
25 for controlling ice crystal growth in ice-cream. This document also describes a process for extracting a polypeptide composition from intercellular spaces of plants by infiltrating leaves with an extraction medium without rupturing the plant cells.

30

WO 94/03617 discloses the production of AFPs from yeast and their possible use in ice-cream. WO 96/11586 describes fish AFPs produced by microbes.

- 5 WO 96/39878 suggests the production of AFP containing frozen compositions by leaving out the hardening step during the freezing process. Suitable storage temperatures are from -10 to -20°F (-23.3 - -28.9°C) and may even be as high as 10°F (-12.2°C).

10

- Up till now, however the use of AFPs has not been applied to commercially available food products. One reason for this is that up till now it has proved difficult to reproducibly produce a frozen food product having the
15 desired texture and eating characteristics.

- The present invention aims at providing solutions to these problems. In particular the invention aims at providing frozen food products with improved mouthfeel and/or taste.
20 Furthermore the invention provides a convenient and cheap process for storing frozen food products containing AFPs.

- Surprisingly it has been found that AFPs can conveniently be incorporated in frozen food products to result in the
25 desired product and process properties if the frozen food product is after preparation stored at a moderate freezing temperature.

- Accordingly in a first aspect the invention relates to a
30 method of storing a frozen food product containing AFPs after preparation, whereby the storage temperature is maintained at from -2.0°C to -12.0°C.

Although applicants do by no means wish to be bound by any theory it is believed that the explanation of the invention is as follows: If food products without AFPs are stored for
5 prolonged periods at relatively high temperatures, normally relatively large ice-crystals are formed throughout the product leading to unacceptable eating characteristics. To avoid this, frozen food products are usually stored at relatively low temperatures say -18°C or lower. A
10 disadvantage of these storage temperatures is however that the frozen products are relatively hard and have a less than optimal flavour release in the mouth if they are eaten at the storage temperature. Surprisingly it has been found that if AFPs are included in food products to be frozen
15 this generally leads to a favourable change in ice-recrystallisation properties allowing the storage of the frozen product at relatively high temperatures and thereby improving the texture and taste if they are eaten at storage temperatures.
20
Frozen products according to the invention have and improved texture and flavour e.g. better creaminess, smoothness, warmth of eating, improved aroma, flavour release etc.
25
Another advantage of relatively high storage temperatures is that freezers can be used with a lower energy consumption and with a reduced tendency of ice-formation in the freezer.
30
Frozen food products of the invention may be any food product which can be stored and/or eaten in the frozen

state. Examples of frozen food products which may contain AFP are processed food products such as for example frozen bakery products e.g. doughs, batters, cakes etc., frozen culinary products for example soups, sauces, pizzas, frozen vegetable products such a compote, mashed potato, tomato paste etc. A very preferred food product according to the invention is a frozen confectionery product.

For the purpose of the invention the term frozen confectionery product includes milk containing frozen confections such as ice-cream, frozen yoghurt, sherbet, sorbet, ice milk and frozen custard, water-ices, granitas and frozen fruit purees. Especially preferred products of the invention are ice-cream and water-ice.

Preferred storage temperatures of the frozen food are from -6.0°C to -12.0°C ., most preferred from -10.0°C to -12.0°C .

The storage temperature of the frozen food refers to the temperature at which the product is maintained after final preparation. A convenient process for the preparation of a frozen product of the invention, especially a frozen confectionery product involves the mixing of the ingredients at a relatively high temperature (e.g. above the freezing point of water, for example at ambient temperature) followed by cooling and freezing. During freezing (optional) aeration may take place e.g. to an overrun of 50 to 250%. Generally the freezing involves a prefreezing step, e.g. in an scraped surface heat exchanger e.g. to a temperature of from -2 to -10°C , followed by filling the prefrozen product into containers followed by

optional hard-freezing e.g. to the storage temperature of the invention.

Frozen products of the invention can be stored at the
5 temperature of the invention for prolonged periods e.g.
from 1 day to 5 years, mostly somewhere between 1 week and
6 months, mostly 2-10 weeks. Suitably frozen products are
stored in a freezer cabinet which is maintained at the
temperature of the invention. These freezer cabinets can be
10 any freezing cabinet which is used for the storage of
frozen food products e.g. in shops or in mobile freezing
cabinets. In shops generally the freezer cabinets are
display cabinets, whereby either part of the cabinet is
transparent or part of the cabinet is open to allow a view
15 of the frozen products.

Applicants have found that the AFPs for use in the process
of the invention can come from a variety of sources.

One possible source of AFP materials is fish. Examples of
20 fish AFP materials are AFGP (for example obtainable from
Atlantic cod, Greenland cod and Tomcod), Type I AFP (for
example obtainable from Winter flounder, Yellowtail
flounder, Shorthorn sculpin and Grubby sculpin), Type II
AFP (for example obtainable from Sea raven, Smelt and
25 Atlantic herring) and Type III AFP (for example obtainable
from Ocean out, Atlantic wolffish, Radiated shanny, Rock
gunnel and Laval's eelpout). A preferred example of the
latter type is described in WP 97/02343.

30 Another possible source of AFP material are invertebrates.
Also AFPs may be obtained from Bacteria.

A third possible source of AFP material are plants.

Examples of plants containing AFPs are garlic-mustard, blue wood aster, spring oat, winter cress, winter canola, Brussels sprout, carrot, Dutchman's breeches, spurge, 5 daylily, winter barley, Virginia waterleaf, narrow-leaved plantain, plantain, speargrass, Kentucky bluegrass, Eastern cottonwood, white oak, winter rye, bittersweet nightshade, potato, chickweed, dandelion, spring and winter wheat, triticale, periwinkle, violet and grass.

10

Both natural occurring species may be used or species which have been obtained through genetic modification. For example micro-organisms or plants may be genetically modified to express AFPs and the AFPs may then be used in 15 accordance to the present invention.

Genetic manipulation techniques may be used to produce AFPs as follows: An appropriate host cell or organism would be transformed by a gene construct that contains the 20 desired polypeptide. The nucleotide sequence coding for the polypeptide can be inserted into a suitable expression vector encoding the necessary elements for transcription and translation and in such a manner that they will be expressed under appropriate conditions (e.g. in proper 25 orientation and correct reading frame and with appropriate targeting and expression sequences). The methods required to construct these expression vectors are well known to those skilled in the art.

30 A number of expression systems may be utilised to express the polypeptide coding sequence. These include, but are not limited to, bacteria, yeast insect cell systems, plant cell

culture systems and plants all transformed with the appropriate expression vectors.

A wide variety of plants and plant cell systems can be
5 transformed with the nucleic acid constructs of the desired polypeptides. Preferred embodiments would include, but are not limited to, maize, tomato, tobacco, carrots, strawberries, rape seed and sugar beet.

- 10 For the purpose of the invention preferred AFPs are derived from fish or plants. Especially preferred is the use of fish proteins of the type III, most preferred HPLC 12 as described in our WO 97/02343. Another preferred AFP is
15 derived from carrots such as described in our non-prepublished case PCT/EP97/06181 or from grass as disclosed in our non-prepublished PCT/EP97/03634.

For some natural sources the AFPs may consist of a mixture of two or more different AFPs.

20

Preferably those AFPs are chosen which have significant ice-recrystallisation inhibition properties. This can be measured in accordance to the method of Example III.

- 25 Preferably AFPs in accordance to the invention provide an ice particle size upon recrystallisation -as measured in accordance to the examples- of less than 20 μm , more preferred from 5 to 15 μm .

- 30 Preferably the level of solids in the frozen food product (e.g. sugar, fat, flavouring etc.) is more than 2 to 3 wt%, more preferred from 4 to 70 wt%.

Very conveniently the products of the invention are packed in closed containers (e.g. cartons, bags, wrappers, boxes, plastic containers etc.). For single portions the pack size will generally be from 10 to 1000 g. For multiple portions pack sizes of up to 500 kg may be suitable. Generally the pack size will be from 10 g to 5000 g.

As indicated above the preferred products wherein the AFPs are used are frozen confectionery product such as ice-cream or water-ice. Preferably the level of AFPs is from 0.0001 to 0.5 wt% based on the final product, more preferred 0.01 to 0.4 wt%, most preferred 0.05 to 0.3 wt%.

The aspect ratio of ice-crystals in compositions which are stored according to the invention is preferably maintained at a level of less than 2.0, e.g. from 1.0 to 1.9. The aspect ratio of ice-crystals is defined as the average ratio of the length and the breadth of the ice-crystals. An aspect ratio of less than 2.0 corresponds to roundish ice-crystals, which are not elongated in shape. The aspect ratio of crystals can be determined by any suitable method.

Example I

A pre-mix for preparing ice-cream was made by mixing:

5 Ingredient	% by weight		
	A	B	C
Skimmed milk powder		10.00	
sucrose		13.00	
maltodextrine (MD40)		4.00	
10 Locust bean gum		0.14	
butter oil		8.00	
monoglyceride (palmitate)		0.30	
vanillin		0.01	
AFP (Type III HPLC-12)	0.01	0.01	none
15 water		balance	

The mixes can be used in the preparation of a ice-cream by homogenisation at 2000 psi and 65°C followed by ageing over night at 5 C. The mix is frozen using a freezer (MF50 SSHE
 20 Technohoy fitted with a solid dasher rotating 240 rpm) The extrusion temperature is -4.5°C, the overrun is 110%. Products A and C are then frozen at -12.0°C and stored for 3 weeks. Product B is frozen at -18°C for 3 weeks. The products are tasted directly after removing from the
 25 freezer.

After 3 weeks storage composition A according to the invention had a markedly better taste and creaminess than the control sample B. Also composition A had a markedly
 30 better texture than composition C.

Example II

An ice-cream was prepared of the following formulation:

5	Ingredient	% by weight
	Skimmed milk powder	10.00
	sucrose	13.00
	maltodextrine (MD40)	4.00
	Locust bean gum	0.14
10	butter oil	8.00
	monoglyceride (palmitate)	0.30
	vanillin	0.01
	AFP (Carrot AFP)	0.01 or none (control)
	water	balance

15

The method of preparation was as in example I.

Carrot AFP was added in the form of an amount of carrot juice obtained from cold acclimatised winter carrots as follows: Root extract from cold acclimatised carrot roots was prepared by scrubbing freshly pulled cold acclimatised carrots in cold water. The tops are removed and the juice extracted employing a domestic juice extractor (Russell Hobbs, model no 9915). The juice was frozen in 1 litre blocks and stored at -20°C prior to collection for use in ice cream trials.

The amount of carrot juice was chosen such that the amount of carrot AFP (the protein having an apparent molecular weight on SDS-PAGE of 38 kDa showing ice-recrystallisation properties) corresponds to 0.01 wt%.

After two months storage at -10.0°C the composition according to the invention had a markedly better texture and flavour than the control sample. Similarly the composition stored at -10.0°C had a better flavour than the 5 same composition which was stored and tasted at -18.8°C .

Example III

Method of determining whether an AFP possesses ice recrystallisation inhibition properties.

5

Recrystallisation inhibition properties can be measured using a modified "splat assay" (Knight et al, 1988). 2.5 μ l of the solution under investigation in 30% (w/w) sucrose is transferred onto a clean, appropriately labelled, 16 mm
10 circular coverslip. A second coverslip is placed on top of the drop of solution and the sandwich pressed together between finger and thumb. The sandwich is dropped into a bath of hexane held at -80°C in a box of dry ice. When all sandwiches have been prepared, sandwiches are transferred
15 from the -80°C hexane bath to the viewing chamber containing hexane held at -6°C using forceps pre-cooled in the dry ice. Upon transfer to -6°C , sandwiches can be seen to change from a transparent to an opaque appearance. Images are recorded by video camera and grabbed into an
20 image analysis system (LUCIA, Nikon) using a 20x objective. Images of each splat are recorded at time = 0 and again after 30-60 minutes. The size of the ice-crystals in both assays is compared. If the size at 30-60 minutes is similar only moderately (less than 10%) increased compared to the
25 size at $t=0$, this is an indication of good ice-crystal recrystallisation properties.

Also if the ice-crystal size number (average length) is less than 20 μm , preferably from 5-15 μm this is a sign of
30 good ice-crystal recrystallisation inhibition properties. The ice-crystal size can conveniently be determined by

highlighting the crystals manually and drawing around the perimeter. Images of the highlighted crystals can then be measured using image analysis software which counts the number of pixels to complete the longest straight line (length), shortest straight line (breadth) and the aspect ratio (length/breadth). The number average length is used as particle size.

Claims

1. A method of storing a frozen food product containing AFPs after preparation, whereby the storage temperature is maintained at from -2.0°C to -12.0°C .
2. A method according to claim 1, wherein the storage temperature is from -10.0°C to -12.0°C .
3. A method according to claim 1, wherein the frozen food product is a frozen confectionery product comprising from 0.0001 to 0.5 wt% of AFPs.
4. A method according claim 1, wherein the product is stored at a temperature from -2.0°C to -12.0°C for a period of 1 day to 5 years.
5. A method according to claim 4, wherein the product is stored at a temperature from -2.0°C to -12.0°C for a period of 1 week to 6 months.
6. Frozen food product stored according to the method of claim 1.
7. Frozen food product according to claim 6 being a frozen confectionery product.
8. Frozen food product according to claim 6 wherein the AFP is derived from plants.

9. Frozen food product according to claim 6 packed in closed containers wherein the pack size is from 10 g to 500 kg.

INTERNATIONAL SEARCH REPORT

national Application No
PCT/EP 98/01576

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 A23G9/00 A23G9/02 A23L3/37		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 A23G A23L		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39878 A (THE PILLSBURY COMP.) 19 December 1996	1-7,9
Y	see page 6, line 7 - line 31; claims 1,2,6,8; examples 1,2 ---	8
Y	WO 97 02343 A (UNILEVER) 23 January 1997 see the whole document ---	8
X	R. E. FEENEY ET AL.: "antifreeze proteins" FOOD TECHNOLOGY, vol. 47, no. 1, 1993, CHICAGO, pages 82-90, XP002040501	1,3,6,7
A	see page 87, column 1, paragraph 2 - column 2, paragraph 1 --- -/--	8
<div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex. </div>		
* Special categories of cited documents		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search <div style="text-align: center; font-size: 1.2em;">22 July 1998</div>		Date of mailing of the international search report <div style="text-align: center; font-size: 1.2em;">29/07/1998</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040. Tx. 31 651 epo nl. Fax: (+31-70) 340-3016		Authorized officer <div style="text-align: center; font-size: 1.2em;">Guyon, R</div>

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/01576

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 03617 A (UNILEVER) 17 February 1994 see abstract	1,8
X,P	GB 2 315 753 A (UNILEVER) 11 February 1998 see claims; examples	1-4,6-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

I national Application No

PCT/EP 98/01576

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9639878 A	19-12-1996	US 5620732 A	15-04-1997
		AU 5790496 A	30-12-1996
		CA 2195950 A	19-12-1996
		EP 0783254 A	16-07-1997
WO 9702343 A	23-01-1997	AU 6519096 A	05-02-1997
		EP 0836646 A	22-04-1998
WO 9403617 A	17-02-1994	AU 4571993 A	03-03-1994
GB 2315753 A	11-02-1998	EP 0843010 A	20-05-1998
		AU 3443797 A	20-02-1998
		AU 3621297 A	20-02-1998
		AU 3621397 A	20-02-1998
		AU 3693497 A	20-02-1998
		DE 19732135 A	26-02-1998
		DE 19732136 A	29-01-1998
		WO 9804699 A	05-02-1998
		WO 9804146 A	05-02-1998
		WO 9804147 A	05-02-1998
		WO 9804148 A	05-02-1998
		FR 2751657 A	30-01-1998
		FR 2751513 A	30-01-1998
		GB 2315752 A	11-02-1998
		DE 19732132 A	29-01-1998
		FR 2751514 A	30-01-1998
		GB 2315662 A	11-02-1998
		WO 9822591 A	28-05-1998